European Journal of Pharmacology 789 (2016) 60–67

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Endocrine pharmacology

Additional effect of metformin and celecoxib against lipid dysregulation and adipose tissue inflammation in high-fat fed rats with insulin resistance and fatty liver

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ARTICLE INFO

Article history:
Received 1 March 2016
Received in revised form
5 July 2016
Accepted 7 July 2016
Available online 7 July 2016

Keywords:
Metformin
COX-2 inhibitor
Obesity
Adipose tissue inflammation
Insulin resistance
Fatty liver

ABSTRACT

We investigated the effects of metformin and celecoxib on obesity-induced adipose tissue inflammation, insulin resistance (IR), fatty liver, and high blood pressure in high-fat (HF) fed rats. Male Sprague-Dawley rats were fed with either regular or HF diet for 8 weeks. Rats fed with regular diet were treated with vehicle for further 4 weeks. HF fed rats were divided into 6 groups, namely, vehicle, celecoxib (30 mg/kg/day), metformin (300 mg/kg/day), metformin (150 mg/kg/day), metformin (300 mg/kg/day) with celecoxib (30 mg/kg/day), and metformin (150 mg/kg/day) with celecoxib (15 mg/kg/day) for additional 4 weeks. Increased body weight in HF fed rats was significantly reduced by metformin alone and metformin combined with celecoxib. The increases in the HOMA-IR value and the area under the curve of glucose following an oral glucose tolerance test, systolic blood pressure, and adipocyte size were significantly diminished in treated rats, especially rats undergoing combined treatment. Treatments with either celecoxib or in combination with metformin resulted in a reduction in AT macrophage infiltration and decreases in levels of adipose tissue TNF-α, MCP-1, and leptin levels in high-fat (HF) fed rats. Furthermore, the elevated hepatic triglycerides content was significantly decreased in the combined treatment group compared to that of groups of celecoxib or metformin alone. Celecoxib exerts a synergistic beneficial effect with metformin on and obesity-associated metabolic and cardiovascular disorders in high-fat fed rats.

1. Introduction

Obesity is a major public health concern with an increasing prevalence worldwide (Flier, 2004). Obesity is associated with insulin resistance (IR) and known as the risk factor for hypertension, dyslipidemia, metabolic syndrome (MS), and type 2 diabetes mellitus (T2DM) (Kopelman, 2000).

Chronic inflammation in fat has been reported to play a crucial role in the development of obesity-induced IR and co-morbidities (Wellen and Hotamisligil, 2003; Winer and Winer, 2012). Adipose tissue macrophage infiltration is considered to contribute to obesity-induced inflammation via secretion of a wide variety of adipose tissue-derived pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) (Chawla et al., 2011; Galic et al., 2011). It has been shown to have local effects on white adipose tissue as well as potential systemic effects on other organs, including liver or skeletal muscle, to promote the development of IR (Guilherme et al., 2008; Shoelson et al., 2006).

Metformin is known as first-line therapy in most clinical practice guidelines for T2DM (American Diabetes, 2015; Nathan et al., 2009) and is recommended for treating obese adolescents with MS (Park et al., 2009). Effects of metformin on hepatic gluconeogenesis are attributed to activation AMP-activated protein kinase (AMPK) (Stephenne et al., 2011). Metformin-induced AMPK activation has resulted in a suppression of acetyl-coA carboxylase (ACC), leading to a reduction in hepatic lipid accumulation and restoration of insulin sensitivity (Shaw, 2013). Metformin is suggested to activate AMPK activity in adipose tissue, through phosphorylation of Thr172 in AMPK in human adipose tissue (Boyle et al., 2011). Moreover, activity of hormone-sensitive lipase (HSL)

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http://dx.doi.org/10.1016/j.ejphar.2016.07.012
0014-2999/

that is regulated by reversible phosphorylation of serine residues has been reported to be inhibited by AMPK activation (Arner and Langin, 2014).

Cyclooxygenase-2 (COX-2) is known to contribute to the pathogenesis of obesity-associated inflammation (Ghoshal et al., 2011; Hsieh et al., 2009). a recent research has suggested that COX-2-mediated inflammation contributes to the development of T2DM in some populations (Konheim and Wolford, 2003). Our previous study has also demonstrated that COX-2-mediated inflammation in adipose tissue is crucially involved in obesity-induced IR and fatty liver in high-fat (HF)-diet fed rats (Hsieh et al., 2009). COX-2 upregulation involves activation of nuclear factor-kappa B (NF-kB) along with increased levels of TNF-α which has been reported to trigger lipid peroxidation in non-alcoholic fatty liver disease (NAFLD) (Leclercq et al., 2004; Yu et al., 2006).

In the present study, we hypothesized that COX-2 inhibition in adipose tissue synergistically enhance the therapeutic effect of metformin on obesity-associated lipid dysregulation and adipose tissue inflammation. We examined potential synergistic effect of a COX-2 inhibitor and metformin on improving obesity-associated IR, fatty liver, and high blood pressure in HF fed rats.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (5–6 weeks old) were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan) and housed in the animal center at National Defense Medical Center, which is certified by the Association of Assessment and Accreditation of Laboratory Animal Care (AAALAC). All animals were handled and housed according to the guidelines and manual of the Committee of the Care and Use of Laboratory Animals in this institute.

The rats were fed with regular chow diet (13.1% fat content) (LabDiet® products Rodent Diet 5010, St. Louis, MO 63,144 USA) or a HF diet (45% fat content) (D12451; New Brunswick, NJ 8901 USA) for 8 weeks. Then, over the following 4 weeks, the rats were further divided into 6 groups, namely vehicle, COX-2 inhibitor (Cel) (celecoxib [30 mg/kg/day] by gavage; Pfizer, New York, NY, USA), metformin (Met) (300 mg/kg/day by gavage; Char Deh, Taipei, Taiwan), Met (150 mg/kg/day) MC (celecoxib [15 mg/kg/day]/metformin [150 mg/kg/day]) and 1/2 MC groups (celecoxib [15 mg/kg/day]+metformin [150 mg/kg/day]).

2.2. Blood pressure measurement

Systolic blood pressure (SBP) was measured as previously described (Hsieh, 2004). In brief, the measurement was carried out in conscious rats at the end of weeks 0, 4, 8, and 12 using an indirect tail-cuff method (volume-oscillometric method) with a fully automatic blood pressure monitoring system (UR-5000; UEDA, Tokyo, Japan).

2.3. Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance test was conducted without anesthesia in the morning after a 14-h overnight fast at the end of weeks 0, 4, 8, and 12. Rats were habituated to the oral gavage procedure 2–3 times with vehicle in one week before the test and to minimize stress throughout the procedure. On the study day, the certain amount of glucose solution (2 g/ml/kg) was administered by gavage, following a baseline blood sample was obtained from a tail vein. Following glucose administration, blood samples were collected at 30, 60, 90, and 120 min

2.4. Biochemical analysis

After an overnight fast, whole blood glucose levels were assayed using the glucose oxidase method. Plasma insulin levels were measured using a commercial rat enzyme-linked immunosorbent assay (ELISA) kit,(Mercodia AB, Uppsala, Sweden). TNF-α, MCP-1, and leptin levels in tissues were analyzed with commercial rat ELISA kits (R&D Systems, Minneapolis, MN, USA). Serum alanine transaminase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine, total cholesterol (TC), low-density-lipoprotein cholesterol (LDL-C), and triglyceride levels were measured using Randox Reagent kits (Randox Laboratories, Ltd., Antrim, UK).

2.5. Calculation

The homeostasis model assessment method (HOMA-IR), an index of systemic IR, was calculated using the following formula before and after treatment in experimental rats: (fasting insulin in μU/ml × fasting glucose in mmol/l)/22.5 (Matthews et al., 1985).

2.6. Immunohistochemistry

Samples of epididymal adipose tissue were fixed in formalin, cut into 4-μm sections, and immunohistochemistry stained using automated stainer (Dako cytomation autostainer; Dako, Glostrup, Denmark). Sections were incubated with ED1 mouse anti-CD68 antibody (Serotec, 1:100; MorphoSys UK, Ltd., Oxford, UK), followed by incubation with goat anti-mouse secondary antibody and development with HRP substrate (Dako REAL EnVision Detection System). Degree of macrophage infiltration in adipose tissue was quantitated by calculating the ratio of nuclei of CD68-positive cells to total nuclei in 20 fields from 3 slides for each individual rat using 6 rats in each group. Adipocyte cross-sectional area was determined for each adipocyte in each field analyzed using Motic Image Plus 2.0 ml software (Ted Pella, Inc., Redding, CA, USA).

2.7. Statistical analysis

Statistical analysis was performed according to the repeated measurements of one-way analysis of variance (ANOVA) incorporating with the Bonferroni test. Pearson's correlation coefficients (r) of the data points from experimental rats were calculated by linear regression. A P value less than 0.05 was considered as a significant difference between groups. Values are expressed as the mean ± S.E.M..

3. Results

3.1. Effects of metformin and/or celecoxib on body weight and food intake, HOMA-IR value, and metabolic parameters

After the drug intervention for 4 weeks, the body weight gain in HF fed rats was reduced in a dose-dependent fashion in presence of metformin and celecoxib (MC/2 and MC; Fig. 1A and B). Caloric intake was significantly intervened in the Met, MC/2, and MC groups, whereas no change was found in the Met/2 and Cel groups (Fig. 1C). A significant reduction in the HOMA-IR value was noted in rats treated with metformin and/or celecoxib, especially in MC group (Fig. 1D). The changes of lipid profile were not significant among experimental groups and plasma BUN and Creatinine levels, the index of renal function were improved in rats treated with full-dose metformin (300 mg/kg/day, Met and MC) groups. The increases in plasma insulin levels and systemic

Please cite this article as: Lu, C.-H., et al., Additional effect of metformin and celecoxib against lipid dysregulation and adipose tissue inflammation in high-fat fed rats with insulin resistance and fatty liver. Eur J Pharmacol (2016), http://dx.doi.org/10.1016/j.ejphar.2016.07.012